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(54) Title: METHOD FOR SELECTING RECOMBINASE VARIANTS WITH ALTERED SPECIFICITY

(57) Abstract: Disclosed are variants of Cre recombinase that have broadened specificity for the site of recombination. Specifically, the disclosed variants mediate recombination between sequences other than the loxP sequence and other lox site sequences on which wild type Cre recombinase is active. In general, the disclosed Cre variants mediate efficient recombination between lox sites that wild type Cre can act on (referred to as wild type lox sites), between variant lox sites not efficiently utilized by wild type Cre (referred to as variant lox sites), and between a wild type lox site and a variant lox site. Also disclosed are methods of recombining nucleic acids using the disclosed Cre variants. For example, the disclosed Cre variants can be used in any method or technique where Cre recombinase (or other, similar recombinases such as FLP) can be used. In addition, the disclosed Cre variants allow different alternative recombinations to be performed since the Cre variants allow much more efficient recombination between wild type lox sites and variant lox sites. Control of such alternative recombination can be used to accomplish more sophisticated sequential recombinations to achieve results not possible with wild type Cre recombinase.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/09154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E.S. TESSMAN ET AL.: "Isolation of protease-proficient, recombinase-deficient recA mutants of Escherichia coli K-12." JOURNAL OF BACTERIOLOGY, vol. 163, no. 2, August 1985 (1985-08), pages 688-695, XP000923354	53-63
Y	the whole document	1-49
X	PARSONS R L ET AL: "FUNCTIONAL ANALYSIS OF ARG-308 MUTANTS OF FLP RECOMBINASE" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 265, no. 8, 1990, pages 4527-4533, XP000907690	53-63
Y	ISSN: 0021-9258 the whole document	1-49
X	J-W. CHEN ET AL.: "Functional analysis of box I mutations in yeast site-specific recombinases Flp and R: Pairwise complementation with recombinase variants lacking the active site tyrosine." MOLECULAR AND CELLULAR BIOLOGY, vol. 12, no. 9, September 1992 (1992-09), pages 3757-3765, XP000923368	53-63
Y	the whole document	1-49
X	ZHANG Y ET AL: "INDUCIBLE SITE-DIRECTED RECOMBINATION IN MOUSE EMBRYONIC STEM CELLS" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 24, no. 4, 1996, pages 543-548, XP000907688	53-63
Y	ISSN: 0305-1048 the whole document	1-49
X	GAGNETEN S ET AL: "BRIEF EXPRESSION OF A GFPCRE FUSION GENE IN EMBRYONIC STEM CELLS ALLOWS RAPID RETRIEVAL OF SITE-SPECIFIC GENOMIC DELETIONS" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 25, no. 16, 1997, pages 3326-3331, XP000907689	53-63
Y	ISSN: 0305-1048 the whole document	1-49

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/09154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. PAN ET AL.: "Mechanism of cleavage and ligation by FLP recombinase: classification of mutations in FLP protein by In Vitro complementation analysis." MOLECULAR AND CELLULAR BIOLOGY, vol. 13, no. 6, June 1993 (1993-06), pages 3167-3175, XP000925153	53-63
Y	the whole document ---	1-49
A	A. WIERZBICKI ET AL.: "A mutational analysis of the bacteriophage P1 Recombinase Cre." JOURNAL OF MOLECULAR BIOLOGY, vol. 195, no. 4, 20 June 1987 (1987-06-20), pages 785-794, XP000923380 cited in the application the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/09154

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-49, 53-63

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/09154

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1-49, 53-63

Remark on Protest

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-49, 53-63

Method for identifying variant recombinases that mediate recombination at variant recombination sites as defined according to claim 1, variant recombinases identified by the method of claim 1, nucleic acid encoding therefor and cell containing said nucleic acid.

2. Claims: 50-52

Method for cloning large DNA fragments according to claim 50

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12N15/52 C12N9/00 C12Q1/25		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N C12Q		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, PAJ, WPI Data, STRAND, BIOSIS, EMBASE, MEDLINE, CAB Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E.S. TESSMAN ET AL.: "Plaque color method for rapid isolation of novel recA mutants of Escherichia coli K-12: New classes of protease-constitutive recA mutants" JOURNAL OF BACTERIOLOGY, vol. 163, no. 2, August 1985 (1985-08), pages 677-687, XP000923356	53-63
Y	the whole document	1-49
X	HARTUNG M ET AL: "CRE MUTANTS WITH ALTERED DNA BINDING PROPERTIES" JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 273, 1998, pages 22884-22891, XP000907691 ISSN: 0021-9258	53-63
Y	the whole document	1-49
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input type="checkbox"/> Patent family members are listed in annex.		
° Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
Date of the actual completion of the international search 4 September 2000		Date of mailing of the international search report 04.12.2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Hix, R